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CNS
- 4) preparing extraction buffer without salt identified as buffer B,
 - 5) preparing extraction buffer with salt identified as buffer C,
 - 6) preparing cytoplasmic extraction clarification buffer identified as buffer D,
 - 7) suspending and maintaining the cells in buffer A for approximately 15 min, *whereas buffer A conc*
 - 8) centrifuging at approximately 2,000 rpm for approximately 5 min at approximately 4 °C,
 - 9) removing the upper cytoplasmic supernatant fraction and clarifying this fraction by adding *cytoplasmic extraction clarif. buff* buffer D *when buff D conc* at approximately 4 °C followed by centrifuging at approximately 13,000 rpm for approximately 15 min,
 - 10) removing the top clear supernatant, quick freezing on dry ice and storing at approximately -86 °C until assayed,
 - 11) washing the bottom nuclear fraction from step 8 with buffer B *extr. buff w/ salt when Buff B or B* and centrifuging at approximately 2,000 rpm for approximately 5 min at approximately 4 °C,
 - 12) suspending the pelleted nuclei in buffer C *cyt buff w salt* on ice,
 - 13) tapping the mixture of step 12 for approximately 45 min to extract the nuclear proteins,
 - 14) centrifuging the mixture of step 13 at approximately 13,000 rpm for approximately 15 min at approximately 4 °C,
 - 15) removing the top clear supernatant, quick freezing on dry ice in aliquotes and storing at approximately -86 °C,
 - 16) storing the remaining bottom nuclear fraction containing nucleic acids from step 14 at approximately -86 °C,
 - 17) performing DNA extraction,

Sub C1 3. (Newly submitted) The method of claim 2 wherein the step of performing DNA extraction comprises the steps of:

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- 1) adding buffer containing Tris-HCl, EDTA, NaCl and SDS to the nuclear fraction of step 16,
 - 2) adding RNAase A followed by proteinase K for approximately 2 hours at approximately 37 °C with gentle tapping,
 - 3) mixing with an equal volume of Tris-HCl and EDTA buffer saturated phenol,
 - 4) centrifuging at approximately 3,000 rpm for approximately 10 min, and collecting the upper aqueous phase containing DNA,
 - 5) mixing with equal volume of phenol and chloroform,
 - 6) centrifuging at approximately 3,000 rpm for approximately 10 min, and collecting the upper aqueous phase containing DNA,
 - 7) mixing with an equal volume of chloroform/isoamyl alcohol prepared at a ratio of 96:4,
 - 8) centrifuging at approximately 3,000 rpm for approximately 10 min,
 - 9) collecting the upper aqueous phase containing DNA and precipitating using a salt and ethanol,
 - 10) centrifuging at approximately 13,000 rpm for approximately 30 min,
 - 11) removing the upper liquid phase and air drying the bottom DNA pellet,
 - 12) dissolving the air dried DNA pellets in Tris-HCl and EDTA buffer.

Sub C1 4. (Newly submitted) The method of claim 2 in which after step 15 further includes the step of analyses of the biologically active transcription factors comprising the steps of:

- 1) synthesizing single stranded oligonucleotides and annealing with the complimentary strand,
- 2) preparing probes by radiolabeling annealed oligonucleotide with γ -³²P-ATP, T4 polynucleotide kinase, and kinase buffer,

- 3) filling in the 5' over-hang ends with Klenow, Klenow buffer and 0.15 mM each of dATP, dCTP, dGTP, and dTTP,
- 4) purifying the labeled oligonucleotides from the unincorporated nucleotides,
- 5) precipitating the labeled, purified oligonucleotides with ethanol,
- 6) resuspending the labeled oligonucleotide probes in sterile Tris-HCl and EDTA buffer,
- 7) performing sequence specific DNA-protein binding analysis by gel electrophoretic mobility shift assay,
- 8) treating the protein extract with specific antibodies prior to the addition of γ -³²P-ATP labeled oligonucleotide probe for supershift assays,
- 9) separating the DNA-protein bound complexes on a acrylamide with bis gel,
- 10) vacuum drying the gel with heat and autoradiography.

5. (Newly submitted) The method of claim 2 wherein the step of preparing cell lysis buffer identified as buffer A comprises:

preparing 20 mM Hepes, pH 7.9, 10 mM NaCl, 3 mM MgCl₂, 0.1% NP-40, 10% glycerol, 0.2 mM EDTA, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 µg/ml antipain, 1 µg/ml leupeptin.

6. (Newly submitted) The method of claim 2 wherein the step of preparing extraction buffer without salt identified as buffer B comprises:

preparing 20 mM Hepes, pH 7.9, 0.2 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 µg/ml antipain, 1 µg/ml leupeptin.

7. (Newly submitted) The method of claim 2 wherein the step of preparing extraction buffer with salt identified as buffer C comprises: